Temocillin quantification in human serum using a high performance liquid chromatography-tandem mass spectrometry

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Introduction

Temocillin (TMO) is a beta-lactam antibiotic that has recently seen both its usage and associated research increased, due to its remarkable resistance to beta-lactamases. [1] Measuring TMO serum concentrations can be critically useful for optimal patient management. A method using HPLC coupled to a UV detector has recently been developed and validated [2]. Yet, UV detection being not specific, interferences could occur when assaying TMO in the serum of patients taking multiple medications.

Aim of the study

To develop and validate a new HPLC method coupled to MS-MS detection for the analysis of temocillin in human serum.

Methods

Methanol protein precipitation was used as the extraction method.
- TMO calibration standards: 1 to 500 µg/mL.
- TMO quality controls: 5 to 450µg/mL.
- Internal standard: Ticarcillin (TIC) - final concentration 160µg/mL.

Sample preparation

200µL of serum + 32µL TIC(1mg/mL) + 200µL (serum + TMO)

Equipment

HPLC (Alliance 2796 Waters) MS-MS
- Column: Xbridge phenyl, 5µm, 2.1x100mm
- Mobile phases: A: H2O+0.1%HCOOH, B: ACN + 0.1% HCOOH
- Flow rate: 0.3 mL/min
- Injection Volume: 10µL
- Gradient elution
- Carryover by concentration
- Recovery is high, precise, reproducible, and is minimally affected by concentration

Results

Linearity

Linearity of HPLC MS-MS detection of temocillin: Peak area ratio (TMO/IS) over the assay concentration range 1- 500 µg/mL

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.993</td>
<td>0.996</td>
<td>0.991</td>
<td>0.993</td>
</tr>
</tbody>
</table>

Standard deviation: 0.0025

Coefficient of variation (CV%): 25

Accuracy

- Mean estimated concentration µg/mL (n=5)
- Precision (CV%): Accuracy (MRPE %)
- Precision (CV%): Accuracy (MRPE %)

<table>
<thead>
<tr>
<th>TMO</th>
<th>LLOQ</th>
<th>1LQC</th>
<th>2LQC</th>
<th>3LQC</th>
<th>4MOC</th>
<th>5MOC</th>
<th>2HQG</th>
<th>4HQG</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.48</td>
<td>1.07</td>
<td>5.17</td>
<td>5.07</td>
<td>7.69</td>
<td>13.06</td>
<td>7.21</td>
<td>6.85</td>
<td>6.48</td>
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<tr>
<td>460.18</td>
<td>15.72</td>
<td>13.62</td>
<td>7.05</td>
<td>13.06</td>
<td>11.68</td>
<td>8.88</td>
<td>2.26</td>
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</tr>
</tbody>
</table>

HQC 450

LLOQ, lower limit of quantification
- LQC, low quality control
- MQC, medium quality control
- HQC, high quality control
- TMO, therapeutic cut-off
- CV, coefficient of variation; MRPE, Mean relative prediction error

Recovery

- Mean % recovery
- Standard deviation
- Coefficient of variation (CV%)

<table>
<thead>
<tr>
<th>TMO</th>
<th>LQC 5</th>
<th>LQC 25</th>
<th>MOC 250</th>
<th>HQC 450</th>
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<tbody>
<tr>
<td>85.80</td>
<td>89.15</td>
<td>90.96</td>
<td>99.40</td>
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<tr>
<td>6.90</td>
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<td>8.04</td>
<td>11.92</td>
<td>7.54</td>
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</tbody>
</table>

Carry-over

- Peak areas of TMO
- Peak areas of blank sample (methanol)
- Peak areas of LLOQ (1µg/mL)

<table>
<thead>
<tr>
<th>LLOQ 1</th>
<th>LQC 1</th>
<th>LQC 25</th>
<th>MOC 250</th>
<th>HQC 450</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.69</td>
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<td>907.25</td>
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<td>296.91</td>
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<td>6.63</td>
<td>11.55</td>
<td>10.73</td>
<td>14.56</td>
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</tbody>
</table>

References


Conclusion

This is the first report describing the quantification of temocillin by HPLC-MS/MS. This method proved fast, specific, and sensitive enough for determining temocillin levels in serum. It could be used for both pharmacokinetic studies and therapeutic monitoring purposes and should avoid any interference with other medications taken by the patients.

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